Appendix I: Publications which reflect the level of skill in the art regarding the identification of conservative sequence modifications which do not remove antigen binding

#	Author	Citation	Comments
1	Brummell *et al.*	(1993) Biochem. 32:1180-1187	Brummell *et al.* used site-directed mutagenesis to examine the binding site of antibodies specific for *Salmonella*. Specifically, the CDR3 heavy chain domain was selected for study and a total of ninety (90) mutants were produced and screened by affinity electrophoresis / Western blots. Those of particular interest were further characterized by enzyme immunoassay and thermodynamic characterization by titration microcalorimetry. Brummell *et al.* found that antigen binding "was retained in a wide range of mutants with only one residue, Gly^{102H}, being irreplaceable."
2	de Wildt *et al.*	(1997) Prot. Eng. 10:835-841	de Wildt *et al.* randomized amino acids in the CDR3 heavy chain regions of anti-UA1 protein antibodies using routine techniques, such as phage display and ELISA screening. de Wildt *et al.* found that "a high frequency (10%) of the randomized mutants in the unselected library were able to bind the [target protein,] U1A protein."
3	Komissarov *et al.*	(1997) J. Biol. Chem. 272:26864-268701	Komissarov *et al.* produced twenty-five site-specific mutants of an anti-oligo(dT) antibody fragment by standard site-directed mutagenesis techniques and examined the role of amino acid residues within the heavy chain CDR3 domain with respect to antigen binding. Komissarov *et al.* found that "[a]ll mutations in the middle of HCDR3 led to either abolished or diminished affinity. Tyr^{101} likely participates in hydrogen bonding, while Tyr^{104} and Tyr^{105} may be involved in aromatic-armatic interactions with the ligand. The residues Arg^{102} and Pro^{103} were not as critical as the tyrosines."
4	Hall *et al.*	(1992) J. Immunol. 149:1605-1612	Hall *et al.* cloned the genes encoding the heavy and light chain variable region genes of the 3-14-9 mAb which binds aminophenyl-β-N-acetylglucosaminide (AZO) and generated Abs that carry mutations within the light chain genes by site-directed mutagenesis and investigated the effects of those mutations with respect to antigen binding. They found that a single change (Gly to Phe) at position 91 within the CDR3 abolished both

			idiotype expression and antigen binding and a chainge (Ala to Thr) at position 25 allowed idiotype expression to some extent but significantly reduced binding activity to AZO.
5	Kelley and O'Connell	(1993) Biochem. 32:6862-6835	The authors probed the relative contribution of polar and nonpolar interactions to antibody-antigen interactions by measuring the effect of single amino acid substitutions in a humanized anti-p185^{HER2} antibody on the thermodynamics of antigen binding. The results show that the aromatic ring of antibody Tyr residues may contribute other interactions to antigen binding, such as aromatic hydrogen bonding, in addition to the contribution from the hydrophobic effect.
6	Adib-Conquy *et al.*	(1998) Int. Immunol. 10:341-346	Site-directed mutagenesis was applied to the heavy chain CDR3 domain of a Fab fragment of a murine IgM Ab. Results showed that, a more hydrophilic composition of the HCDR3 conferred to the Fab a polyreactive profile, whereas the reactivity of a Fab containing a hydrophobic HCDR3 rich in aliphatic residues was more restricted. Further, prolines played an important role in this context, because their presence at each end of the HCDR3 restricted the Ab's flexibility and, in turn, its reactivity.
7	Beers *et al.*	(2000) Clin. Can. Res. 6:2835-2843	Beers *et al.* used random CDR mutagenesis (nine residues of the HCDR3 were randomly mutagenized) to obtain mutants of MR1(Fv), a single-chain recombinant immuntoxin that targets EGFRvIII, with increased affinity and increased immunotoxin activity. All mutations were located at amino acids 98 an 99.